Possible roles of the peripheral vagal nerve in histamine-induced bronchoconstriction in guinea-pigs

H. Inoue, H. Alzawa, N. Miyazaki, T. Ikeda, N. Shigematsu

**ABSTRACT:** Although the importance of the vagal nerve in the pathogenesis of bronchial asthma has been reported, its precise contribution is still not fully understood. To shed more light on this area, we evaluated the possible contribution of vagal reflex in histamine-induced bronchoconstriction (HIB), and decided the site of action of histamine on the vagal nerve.

For this purpose, we studied the effects of the bilateral cervical vagotomy, hexamethonium (2 mg·kg⁻¹) or tetrodotoxin (0.5 mg·kg⁻¹) on HIB (8 μg·kg⁻¹ iv) in anesthetized and mechanically-ventilated guinea-pigs. We also studied whether or not atropine (1 mg·kg⁻¹) decreases HIB after vagotomy, including either the treatment of hexamethonium or tetrodotoxin. Airway responses were assessed by measurement of pulmonary resistance.

The following results were obtained: 1) the response to histamine was significantly enhanced by the vagotomy, hexamethonium or tetrodotoxin; 2) propranolol increased HIB, and HIB was further enhanced by the vagotomy in the animals treated with propranolol; 3) atropine significantly suppressed HIB after the vagotomy, hexamethonium or tetrodotoxin.

These results suggest that the postganglionic vagal nerve plays an excitatory role in HIB through the release of acetylcholine from the nerve terminals. It is also suggested that the vagal reflex mainly exhibits an inhibitory role in the HIB of guinea-pigs, presumably by the action of the nonadrenergic inhibitory nervous system.

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The precise mechanisms involved in airway hyperresponsiveness are not yet fully understood. However, it has been considered that the vagal nerve plays an important role in the pathogenesis of airway hyperresponsiveness [1–5]. It has been suggested that an augmented vagal reflex may be involved in the development of the increased responsiveness of the airways, since the stimuli such as histamine and allergen to the vagal sensory nerve cause bronchoconstriction which can be inhibited by sectioning/cooling of the vagal nerves or by a cholinergic antagonist [6–9]. However, it was also reported that a vagotomy does not suppress the bronchoconstriction induced by histamine [10, 11], and therefore the possible role of vagal reflex is still controversial.

Furthermore, it has been suggested that the vagal nerve may participate locally in the bronchoconstriction [12]. Namely, the stimulation of afferent vagal nerve endings may result in an "axon reflex" by releasing tachykinins which cause bronchoconstriction. It is also possible that histamine may directly stimulate the efferent vagal nerve terminals and cause bronchoconstriction through the release of acetylcholine [13–15]. However, the mechanism of activation of the peripheral vagal nerve by histamine remains to be clarified.

The first aim of this study was to evaluate the relative contributions of the vagal reflex involved in the airway response to histamine. The second was to determine the site of action of histamine on the peripheral vagal nerve. The third was to clarify the inhibitory mechanism involved in the vagal reflex.

**Methods**

**Procedure**

Guinea-pigs, of either sex, weighing 500–900 g, were anaesthetized with pentobarbital sodium (50 mg·kg⁻¹, i.p.), and ventilated with a respirator (Harvard Apparatus, Model no. 680) through a tracheal cannula at a constant tidal volume (7–8 ml·kg⁻¹) and a rate of 60 breaths·min⁻¹.
A catheter was inserted into a jugular vein for the administration of drugs, and another catheter was inserted into a carotid artery for the measurement of blood pressure using an electric manometer (Nihon Kohden, LPU-0.1). A fluid-filled catheter was inserted into the oesophagus to measure the oesophageal pressure for an evaluation of pleural pressure. The oesophageal catheter was positioned at the point where the maximal amplitude of pleural pressure can be obtained. The bilateral cervical vagal nerves were exposed. Sham surgery was performed in the control group. The animals were placed supine in a body-plethysmograph. Plethysmograph airflow (V) was measured with a Fleisch pneumotachograph (Nihon Kohden, TV-132T) and a differential pressure transducer (Nihon Kohden, TP-602T). The tidal volume (VT) was measured by a differential pressure transducer (Nihon Kohden, TP-603T). The baseline variability of intravenous histamine were reproducible.

Variability of histamine-induced bronchoconstriction (HIB). We studied the effects of three consecutive histamine challenges in the control group in order to determine whether the response to repeated single doses of intravenous histamine were reproducible.

Effect of atropine on HIB. To determine whether atropine can decrease the HIB, we repeated histamine injections three times, once before and then 5 and 30 min after the pretreatment of atropine (1 mg·kg⁻¹, i.v.).

Effect of vagotomy and atropine on HIB. To determine the effects of the cervical vagotomy on HIB, and the effects of atropine on HIB after the vagotomy, we cut the bilateral cervical vagal nerves after the control injection of histamine. We waited 10 min until the RL returned to the initial baseline values after the vagotomy, and measured the histamine response. Thereafter, atropine was administered, and then a histamine injection was repeated 5 min after atropine.

Effect of hexamethonium and atropine on HIB. To determine whether the blockade of the neural transmission in the ganglia has an effect similar to vagotomy, we measured the HIB both before and 5 min after treatment with hexamethonium (2 mg·kg⁻¹, i.v.). We then administered atropine and injected histamine again 5 min after atropine.

Effect of vagotomy on HIB after propranolol. To investigate the contribution of inhibitory effects by adrenergic and nonadrenergic inhibitory nervous system, we measured the HIB both before and 5 min after treatment with propranolol (1 mg·kg⁻¹, i.v.). We then performed a bilateral cervical vagotomy and injected histamine after the vagotomy.

Effect of tetrodotoxin and atropine on HIB. To evaluate the effects of the inhibition of neural transmission, we studied the HIB both before and 5 min after the treatment of tetrodotoxin (0.5 mg·kg⁻¹). We then repeated the histamine injection 5 min after the atropine.

Fig. 1. – Dose-response curve for intravenous histamine on pulmonary resistance (RL) in five guinea-pigs.

We also performed dose-ranging studies on bradykinin in five guinea-pigs, and used 1 μg·kg⁻¹ of bradykinin.

Study design

Three repeated histamine challenges (8 μg·kg⁻¹, i.v.) were performed on each guinea-pig according to the following protocols. Sufficient time was allowed for the RL to return to the initial baseline values after injection. Five animals were used in each experiment.

Effect of atropine on bradykinin-induced bronchoconstriction. To rule out any possibility that the atropine might have anti-histaminic properties, we studied the inhibitory effect of atropine on bradykinin-
induced bronchoconstriction (1 μg·kg⁻¹, i.v.). We injected bradykinin before, and then 5 and 30 min after the atropine.

**Time-matched control on HIB.** It is not possible to exclude that the effect of first treatment, such as vagotomy, hexamethonium, tetrodotoxin or propranolol, could be time dependent, and thus contribute to changes ascribed to second treatment, such as atropine or vagotomy. We studied the HIB with or without the second treatment after the first treatment. In the experiment of time-matched control, we injected a vehicle or did sham surgery instead of the second treatment. Ten animals were used in each group. The values of RL are expressed as percentage of the maximal response after the first treatment.

**Data analysis**

The values of pulmonary resistance are expressed as the percentage of the initial baseline values, except in the time-matched control group. Data are expressed as arithmetic means and standard errors of the means in each experimental series. Differences between the means were determined using Student’s t-test for paired samples. A p value <0.05 was considered to be statistically significant.

**Drugs**

The following drugs were used; pentobarbital sodium (Abbott Lab., North Chicago, IL), histamine diphosphate, atropine sulphate, propranolol hydrochloride, hexamethonium bromide and tetrodotoxin (Sigma Chemical, St. Louis, MO).

**Results**

Histamine injection increased Ptp, decreased V and markedly increased RL in the control guinea-pigs. The changes started at about 3 s after injection. The maximal change of the RL from the baseline values occurred at about 10 s after injection. These changes were transient and returned to baseline values within 3 min. Systemic blood pressure started to fall about 5 s after injection, and returned to the baseline within 3 min (fig. 2). A vehicle injection had no effect on the RL or blood pressure.

**Variability of HIB.** In the control experiments, repeated histamine injections in the same animal resulted in virtually identical airway responses, as shown in table 1.

**Effects of atropine on HIB.** Atropine caused a significant suppression of airway responsiveness to intravenously administered histamine (p<0.01) (fig. 3). Thirty minutes after treatment with atropine, the response to histamine returned to control values (p>0.1). The baseline RL values before, 5 min and 30 min after the atropine were not significantly different.

**Effects of vagotomy and atropine on HIB.** The surgical procedure for the bilateral vagotomy caused transient increases in RL, and the changes in RL returned spontaneously to initial baseline levels. The vagotomy
enhanced the HIB markedly. In five guinea-pigs, the response to histamine was significantly greater than that of the controls after vagotomy \(^*\) \((p<0.05)\). Atropine itself did not change the baseline \(R_L\) \((p>0.1)\). Atropine suppressed the HIB significantly in vagotomized guinea-pigs \((p<0.05)\) (fig. 4).

**Effects of hexamethonium and atropine on HIB.**
The pretreatment with hexamethonium *per se* did not affect the baseline \(R_L\) \((p>0.1)\). However, HIB was significantly enhanced after pretreatment with this chemical \((p<0.01)\) (fig. 5). The decrease in response to histamine by atropine was also observed in the animals treated with hexamethonium \((p<0.01)\).

**Effects of vagotomy on HIB after propranolol.** As shown in figure 6, propranolol increased HIB significantly. Pretreatment with propranolol itself did not show any effects on the baseline \(R_L\) \((p>0.1)\). The maximal change in \(R_L\) by histamine was significantly greater as compared
to the control response after the application of propranolol (p<0.01). A bilateral vagotomy further increased changes in RL by histamine in the animals treated with propranolol (p<0.05).

Effects of tetrodotoxin and atropine on HIB. Tetrodotoxin increased the HIB significantly (fig. 7). The maximal changes in RL by histamine were significantly greater than that of the control after the treatment of tetrodotoxin (p<0.05). The pretreatment of tetrodotoxin itself did not affect the baseline RL (p>0.1) or the blood pressure. The decrease in HIB by atropine was observed in animals treated with tetrodotoxin (p<0.05).

Effects of atropine on bradykinin-induced bronchoconstriction. Three injections of bradykinin evoked reproducible bronchoconstrictions. The maximal (post-injection) RL values were 191±15%, 194±12%, 185±10%. As shown in figure 8, atropine suppressed bradykinin-induced bronchoconstriction significantly (p<0.01). Thirty minutes after the treatment of atropine, the response to bradykinin returned to the control values (p<0.1).

Time-matched control on HIB. The responses to histamine in the atropine-treated animals were significantly less than that in the animals with vehicle after the first treatment with vagotomy. The findings were the same as in the first treatment group with hexamethonium or tetrodotoxin, as shown in table 2. The HIB in the vagotomized animals were significantly greater than that in the animals with sham surgery after pretreatment with propranolol.

### Table 2. Maximal change of RL induced by histamine after first and second treatment

<table>
<thead>
<tr>
<th>Group</th>
<th>1st treatment</th>
<th>2nd treatment</th>
<th>n</th>
<th>RL change</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Vagotomy</td>
<td>Atropine</td>
<td>5</td>
<td>100</td>
<td>93±2</td>
</tr>
<tr>
<td>Group 1</td>
<td>Vagotomy</td>
<td>Atropine</td>
<td>5</td>
<td>100</td>
<td>55±7</td>
</tr>
<tr>
<td>Group 2</td>
<td>Hexamethonium</td>
<td>Atropine</td>
<td>5</td>
<td>100</td>
<td>128±14</td>
</tr>
<tr>
<td>Group 2</td>
<td>Hexamethonium</td>
<td>Atropine</td>
<td>5</td>
<td>100</td>
<td>70±4</td>
</tr>
<tr>
<td>Group 3</td>
<td>Propranolol</td>
<td>Vagotomy</td>
<td>5</td>
<td>100</td>
<td>104±30</td>
</tr>
<tr>
<td>Group 3</td>
<td>Propranolol</td>
<td>Vagotomy</td>
<td>5</td>
<td>100</td>
<td>248±41</td>
</tr>
<tr>
<td>Group 4</td>
<td>Tetrodotoxin</td>
<td>Atropine</td>
<td>5</td>
<td>100</td>
<td>133±9</td>
</tr>
<tr>
<td>Group 4</td>
<td>Tetrodotoxin</td>
<td>Atropine</td>
<td>5</td>
<td>100</td>
<td>58±8</td>
</tr>
</tbody>
</table>

The values are expressed as percentage of the response after first treatment. RL: pulmonary resistance.
Discussion

This study demonstrates that airway response to histamine was enhanced by the bilateral cervical vagotomy or the treatment with hexamethonium or tetrodotoxin. It is also demonstrated that atropine markedly suppressed the response to histamine even after the vagotomy. These results indicate that the vagal reflex may play an inhibitory role in the histamine-induced bronchoconstriction, and that the cholinergic bronchoconstriction may still remain even after the vagotomy.

It has been reported that bronchoconstriction induced by histamine may involve vagal reflex. The bronchoconstriction induced by histamine injection into bronchial arteries or inhalation was suppressed either by cooling or cutting the cervical vagal nerves in dogs [6, 7, 9] and cats [17]. In awake guinea-pigs, it has also been reported that vagotomy attenuates HIB [8]. The importance of the vagal reflex was also suggested by the reduction in HIB by atropine in guinea-pigs [18, 19]. These results suggest that the vagal reflex might be an important factor for development of increased responsiveness of the airways.

In the present study, however, vagotomy did not attenuate but enhanced the HIB, and atropine did suppress HIB even after vagotomy. These findings raise the possibility that the "peripheral vagal nerve" may play a role in the enhancement of HIB, by releasing acetylcholine. The response after vagotomy consists of the direct action to smooth muscle and the enhancement by the peripheral vagal nerve. Atropine eliminates the component of vagally mediated response. This possibility was supported by the results that the treatment with hexamethonium enhanced HIB, and that atropine also suppressed HIB after hexamethonium. Furthermore, atropine suppressed the bronchoconstriction induced by another bronchoconstrictor, bradykinin, as well as histamine. We consider this result rules out the possibility that the suppression of HIB by atropine may be due to an anti-histaminic activity of this agent.

Discrepancy in the present and previous findings on the effects of vagotomy is not fully understood, however, several methodological differences may partly account for it. We used anaesthetized animals under mechanical ventilation. It has been reported that there was less vagally mediated bronchoconstriction in ventilated than spontaneously breathing rabbits [20]. Anaesthesia has also been shown to suppress vagal reflexes [21]. We studied the effects of the cervical vagotomy on HIB using different anaesthesia, alphachloralose and urethan. The results of the experiment with chloralose and urethan were the same as that with pentobarbital (the data were not shown). We consider the difference of anaesthesia had little influence on the present findings.

The present findings are supported by several previous investigations. Shore et al. [9] reported that atropine attenuated HIB after bilateral vagotomy in dogs. Other previous studies reported that the bronchoconstrictor response to histamine was unaffected by either vagal sectioning or cooling in either guinea-pigs [10, 11] or dogs [22-24].

Concerning the sites of action of histamine on the peripheral vagal nerve, there are two possibilities. Firstly, histamine may stimulate efferent vagal nerve terminals to release acetycholine, since atropine suppressed HIB after tetrodotoxin in the present study. Treatment of tetrodotoxin completely blocked the neural transmission of vagal nerve, because electrical vagal stimulation (10 V, 50 Hz, 1 ms, 5s) did not evoke any bronchoconstriction after the administration of tetrodotoxin (data not shown). These results support the possibility that histamine might stimulate efferent nerve terminals directly and release acetycholine. Secondly, stimulation of afferent nerve terminals by histamine may cause a "local reflex" and release acetycholine.

The augmentation of HIB after vagotomy suggests an inhibitory role of a "central vagal reflex", rather than an excitatory role. The adrenergic system may participate in this inhibitory effect, because propranolol enhanced the response to histamine. HIB was further enhanced by the vagotomy in animals treated with propranolol. This result suggests the contribution of the nonadrenergic inhibitory nervous system (NAIS) on the vagal reflex, since NAIS is involved in the vagal trunk [25, 26]. It has recently been reported that NAIS can be reflexly activated in humans [27] and cats [28]. A previous study from our laboratory demonstrated that hexamethonium induces airway hyperresponsiveness in cats treated with atropine and propranolol, suggesting that the blockade of NAIS causes hyperresponsiveness [25].

In conclusion, this study has demonstrated the possibility that the "peripheral vagal nerve", at a site close to an airway, has an excitatory effect independent of the "central vagal reflex" in HIB. It is also suggested that the "central vagal reflex" mainly exhibits an inhibitory role in guinea-pigs in HIB, presumably due to the action of NAIS.

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References


RÉSUMÉ: Quoique l'importance du nerf vague dans la pathogénie de l'asthme bronchique ait été signalée, sa contribution exacte n'est pas encore complètement comprise. Afin d'éclairer davantage cette problématique, nous avons évalué la contribution possible d'un réflexe vagal dans la bronchoconstriction induite par l'histamine (HIB), et recherché le site d'action de l'histamine sur le nerf vague.

Dans cette optique, nous avons étudié les effets de la vasoconstriction cervicale bilatérale, de l'hexaméthonium (2 mg·kg⁻¹) ou de la tetrodotoxine (0.5 mg·kg⁻¹) et l'atropine (1 mg·kg⁻¹) sur l'activité des voies aériennes coronaires. Les résultats suivants ont été obtenus: 1) La réponse à l'histamine est fortement accentuée par la vasoconstriction, l'hexaméthonium ou la tetrodotoxine. 2) Le propranolol a augmenté HIB et HIB a été accentuée davantage par la vasoconstriction chez des animaux traités au propranolol. 3) La différence de façon significative HIB après vagotomie, hexaméthonium ou tetrodotoxine. Les résultats des voies aériennes ont été appréciées par mesure de la résistance pulmonaire.

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Ces résultats suggèrent que le nerf vague post-ganglionnaire joue un rôle excitateur dans HIB par la libération d'acétylcholine à partir des terminaisons nerveuses. L'on suggère également que le réflexe vagal exerce principalement un rôle inhibiteur dans la HIB des voies aériennes, probablement par l'action du système nerveux inhibiteur non adrénergique. Eur Respir J., 1991, 4, 860-866.